Developing a Predictive Capability for Bioluminescence Signatures

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LONG-TERM GOALS

Bioluminescence represents an operational threat to naval nighttime operations because the flow field associated with their motion stimulates naturally occurring plankton. In the littoral, the primary sources of bioluminescence are dinoflagellates, common unicellular plankton that are also known to form red tides. Dinoflagellate bioluminescence is stimulated by flow stress of sufficient magnitude to cause cell deformation, such as in the boundary layers of swimming animals, in separated flow of the wakes of animals, fixed objects, and ships, and in breaking surface waves, leading to spectacular displays of bioluminescence during periods of high dinoflagellate abundance. The oceans can be considered a luminescent minefield where bioluminescence is stimulated by flow disturbance. The bioluminescent signatures of some swimming fish are distinct enough to differentiate species; nocturnally foraging predators may use bioluminescent wakes to locate their prey.

The bioluminescence signature of a moving object depends on the bioluminescence potential of the organisms (related to their species abundance and measured by bathyphotometers), the spatial characteristics of stimulatory flow regions, level of flow stress, and the detectability of the light source from a surface observer based on radiative transfer of the light through the water and surface interface, as well as surface ambient light conditions. We are interested in predicting bioluminescence signatures, specifically in developing the capability to computationally predict levels of flow stimulated bioluminescence. This predictive capability is based on a thorough understanding of the light-emitting characteristics of the source organisms and the measurement of their bioluminescence by ocean

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sensors, and will allow us to explore mitigation strategies that reduce the bioluminescence signature to decrease the threat of detection of moving underwater objects.

OBJECTIVES

An extremely challenging goal is the need to predict the intensity and spatial footprint of bioluminescence signatures of naval relevance. Advances in computational fluid dynamics (CFD) led by PI Hyman make it possible to model the flow around a moving object, and now a new bioluminescence stimulation (BIOSTIM) model developed by PI's Deane and Stokes (Deane and Stokes 2005) provides an initial capability to estimate bioluminescence levels as a function of flow properties, specifically fluid shear stress, which we have previously shown to be the flow property most closely correlated with flow-stimulated bioluminescence in primarily laminar flows (Latz et al. 1994; Latz et al. 2004; Latz and Rohr 1999; Maldonado and Latz 2007).

The primary scientific objective of this project is to couple the BIOSTIM and CFD models to predict bioluminescence signatures associated with a moving object for a given level of bioluminescence potential. In the process we re-evaluated the BIOSTIM model based on the latest evidence; evaluated computational approaches using Reynolds-averaged Navier-Stokes (RaNS) and Direct Numerical Simulation (DNS) solvers, to determine which is more suitable for bioluminescence predictions; and validated the BIOSTIM model with laboratory tests involving independent flow fields that are characterized using CFD models, so that model predictions of bioluminescence intensity can be compared to experimental results. In addition we have evaluated some of the flow agitators used in measuring bioluminescence potential, to determine the effect of chamber design on the measured bioluminescence potential. Finally, we have investigated the cellular regulation of dinoflagellate bioluminescence as a mitigation strategy. In sum, we are working to understand the regulation of dinoflagellate bioluminescence, predict bioluminescence signatures for flow fields of naval interest based on levels of bioluminescence potential, and characterize systems used to measure bioluminescence potential, as contributions to the data products of NAVOCEANO and NRL and future strategies for bioluminescence mitigation.

APPROACH

The current probabilistic model for bioluminescence stimulation (BIOSTIM) (Deane and Stokes 2005) contains three components to allow for: (1) direct stimulation by fluid shear stress, (2) rate-of-change of fluid shear stress, and (3) a memory term to allow for cell desensitization resulting from prolonged exposure to stimulation. The model is based on the fundamental assumption that over any small time interval there is a small but finite chance that a cell will flash, which depends on these three factors. This study considers the case of intense but brief stimulation in which the rate of change of shear stress is high and time scales are sufficiently short to minimize desensitization (von Dassow et al. 2005) and cell memory, greatly simplifying the experiments and analysis required to model the effects of turbulence.

The overall objective of this study is to obtain bioluminescence stimulation data under conditions of high shear stress to feed into the BIOSTIM model, which then is incorporated into CFD models to predict bioluminescence signatures created by bodies traveling in or on the ocean. The most generally applicable simulation techniques are algorithms that solve the unsteady Reynolds-averaged Navier-Stokes (uRaNS) equations and compute the ensemble-averaged velocities, as well as turbulent energy and energy dissipation fields throughout a given flow, allowing an estimation of local (averaged)

turbulent shear stress. The uRaNS algorithm to be used in the proposed task is CFDSHIP-IOWA, a well-documented algorithm (Carrica et al. 2006) previously used by PI Hyman and verified with full-scale tests with many types of naval ships. However, such algorithms cannot resolve the very small scales that are responsible for bioluminescent stimulation. The action of such small-scale turbulence is approximately characterized by the averaged energy dissipation rate – a modeled quantity. In contrast, the BIOSTIM model, as currently written, is most appropriate for use in a Direct Numerical Simulation (DNS) solver. DNS solutions capture all relevant length and temporal scales in the flow including bioluminescence stimulatory scales (these are in the Kolmogorov or inertial range, depending on Reynolds number). To accomplish this, however, the solvers require extremely fine grids – grids that become too large when flow simulation of model-scale vehicles is attempted and far too large to be considered for full-scale naval vehicles. Therefore the new bioluminescence stimulation model developed in Task 2 will accept the ensemble-averaged flow data produced during a practical flow simulation as a means of determining stimulation probability.

The BIOSTIM model is incorporated into a CFD model to predict the proportion of bioluminescence potential as a function of space and time. So model predictions of bioluminescence intensity were based on the stimulation probability and bioluminescence potential. CFD algorithms typically solve the Reynolds-averaged Navier-Stokes (RaNS) equations to compute average velocity and turbulent energy dissipation through a given flow field, from which turbulent shear stress is estimated. Because of the small spatial scales of the organisms and the formulation of the BIOSTIM model, an approach using a Direct Numerical Stimulation (DNS) solver was also used. During the project, grid size has been increased to better capture the relevant spatial and temporal scales in the flow. DNS and RaNS results were compared for the same sized sphere as a representative object. Model predictions were validated using a sting-mounted sphere moving at speeds up to 1 m/s.

WORK COMPLETED

1. The first goal for integrating the computational and experimental activities was to perform direct numerical simulations (DNS) of the flow field associated with a sphere for velocities of 0.5 and 1 m/s, to obtain 2D maps of local fluid shear stress. The current BIOSTIM model was incorporated into the DNS results to predict levels of stimulated bioluminescence; these predictions were compared to existing bioluminescence images of a sting-mounted sphere moving at the same speeds in a flume and imaged with a digital low-light camera.

1.1. Computational work. Work continued on improving the CFD simulation of flow, shear stress and related bioluminescent stimulation in the wake of a sphere. While early computations were encouraging, the stimulation probability showed a much smaller geometry than the images from tow tank measurements. Therefore more recent work improved the sphere solution, and working on flow computations over an ellipsoid, compared computational results to experimental observations. Further details of the computational work are contained in a separate report by PI Hyman (Grant number N0001412WX20034).

1.2. Experimental work. Observations of bioluminescence associated with a towed 6:1 ellipsoid 15 cm long were made in the vertical test chamber. Speeds up to 1.5 m/s were achieved without appreciable unsteady motion of the ellipsoid, with initial tests run for cultures of *L. polyedrum* at a concentration of 10 cells/ml. These observations were compared to computed predictions of bioluminescence for the ellipsoid.

2. Flow agitation characterization. Two different bathyphotometer flow agitators were evaluated in the laboratory using four species of dinoflagellates as a function of volume flow rate and dinoflagelalte concentration, to determine the critical volume flow rate, and the spatial pattern and residence time of flash trajectories. A previous data set using turbulent pipe flow of plankton samples was used to scale bioluminescence intensity by bathyphotometer bioluminescence potential.

3. Biological work. Laboratory experiments with the dinoflagellate *Lingulodinium polyedrum*, a model species for bioluminescence studies, used a pharmacological approach to target specific areas of the signaling pathway involved in the cellular regulation of bioluminescence.

RESULTS

1.1 Model predictions were made for a 32-mm diameter sphere moving at a speed of 1 m/s (Figures 1, 2). DNS results were optimized by increasing grid resolution, so that the latest fine resolution version involved 100 million (10^8) grid points, corresponding to a near-DNS solution with a spatial resolution of 1.5 times the dissipation length scale. The stimulation region extended nearly 2.5 diameters downstream and exhibited a roughly cylindrical shape. The solution suggests that unsteady vortex shedding should lead to a stimulation field slightly large than the sphere diameter, with maximum stimulation probability a small distance downstream of the body. Unfortunately images obtained from tow tank tests were different from the computational predictions.

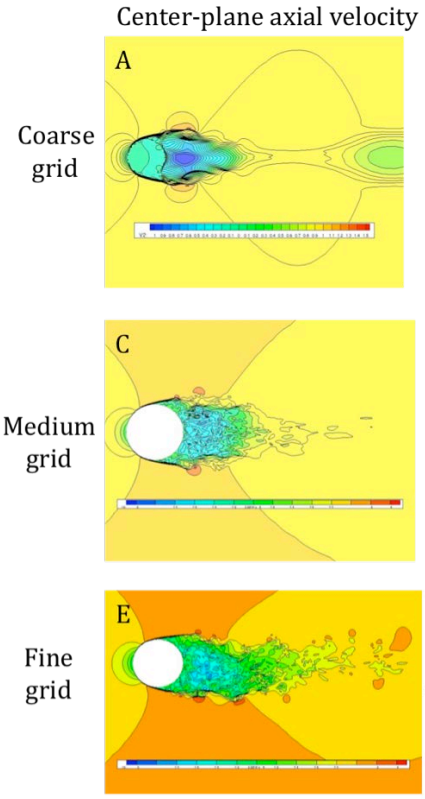

Figure 1. Contour plots for DNS computed center-plane instantaneous axial velocity field of a 32 mm diameter sphere, moving to the left at a speed of 1 m/s. (A) Results for the original coarse resolution grid. (C) Results for a medium resolution 351 x 351 x 601 grid. (E) Results for a fine resolution 351 x 351 x 801 grid. The stimulation region extends nearly 2.5 diameters downstream and exhibits a roughly cylindrical shape. The solution strongly suggests that unsteady vortex shedding should lead to a stimulation field a little larger than the sphere diameter, and that maximum stimulation probability is a small distance downstream of the body.

Due to the limitations of the DNS algorithm, an unsteady Reynolds averaged Navier-Stokes (uRaNS) algorithm was applied. RaNS models are commonly performed in CFD computations; they are effective and accessible, requiring 10 million grid points making it computationally less expensive to run. However, RaNS models do not provide shear stress at the scale of the organism. Thus the BIOSTIM model could not be directly applied. Instead the near-DNS solution was used to calibrate the RaNS solution.

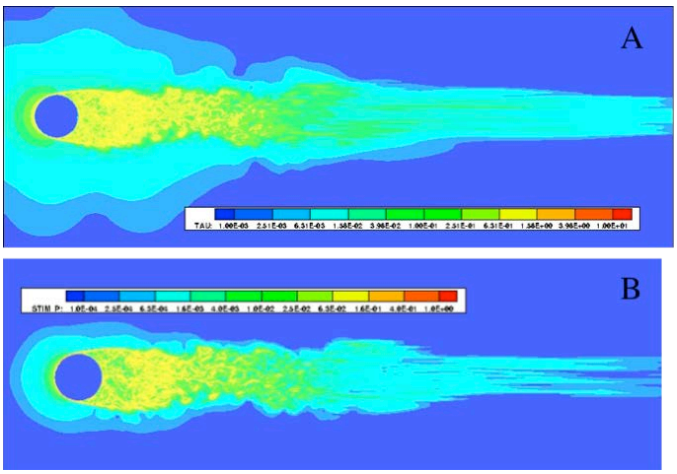

Figure 2. Contour plots for DNS computed stimulation field of a 32 mm diameter sphere, moving to the left at a speed of 1 m/s. (A) Instantaneous local shear stress from the near-DNS solution. (B) Probability of stimulation of bioluminescence (expressed as 1/s), computed from the near-DNS solution using the BIOSTIM model.

If an uRaNS solution for the same sphere is obtained and the shear stress is computed using the computed two-equation k-epsilon/k-omega turbulence model, the results (shear stress along the centerline) shown in Figure 3A are produced. This shear stress field can be used as input to the BIOSTIM model and an estimate of stimulation probability can be obtained. The resulting probability field, integrated along the viewer's line-of-sight, is shown in Figure 3B. Comparison of the shear stress in Figure 2A and stimulation probability in 2B with those in 3A and 3B suggest that the uRaNS solver is not providing a local (to the organism) shear field, but instead a total shear field. If the uRaNS results are corrected using the DNS results, the line-of-sight integrated stimulation probability shown in Figure 3C is obtained.

Knowing the probability of stimulation, bioluminescence can be predicted based on the bioluminescence potential or concentration of organisms. Predictions of the bioluminescence signature of the 32-mm diameter sphere moving at a speed of 1 m/s were based on a *Lingulodinium polyedrum* cell concentration of 32 cells/ml (Figure 4A). Predicted results were compared to actual measurements taken with a 32-mm diameter sphere moving at a speed of 1 m/s (Figure 4B). Predicted bioluminescence was within a factor of 2 of measured results.

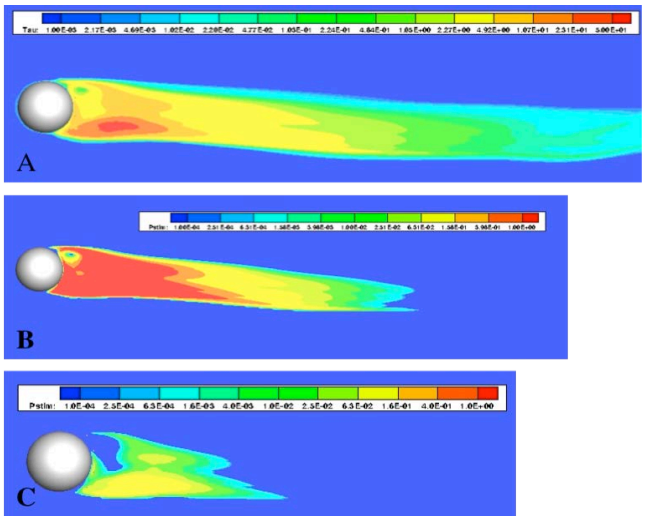

Figure 3. Contour plots for RaNS computed stimulation field of a 32 mm diameter sphere, moving to the left at a speed of 1 m/s. (A) Reynolds-averaged shear stress, based on the energy-containing length scales. (B) Probability of stimulation of bioluminescence (expressed as 1/s) using the BIOSTIM model. (C) Probability of stimulation of bioluminescence (expressed as 1/s) using DNS to calibrate the shear stress. The solution is scaled by sphere diameter, as the energy-containing length scales are proportional to the diameter.

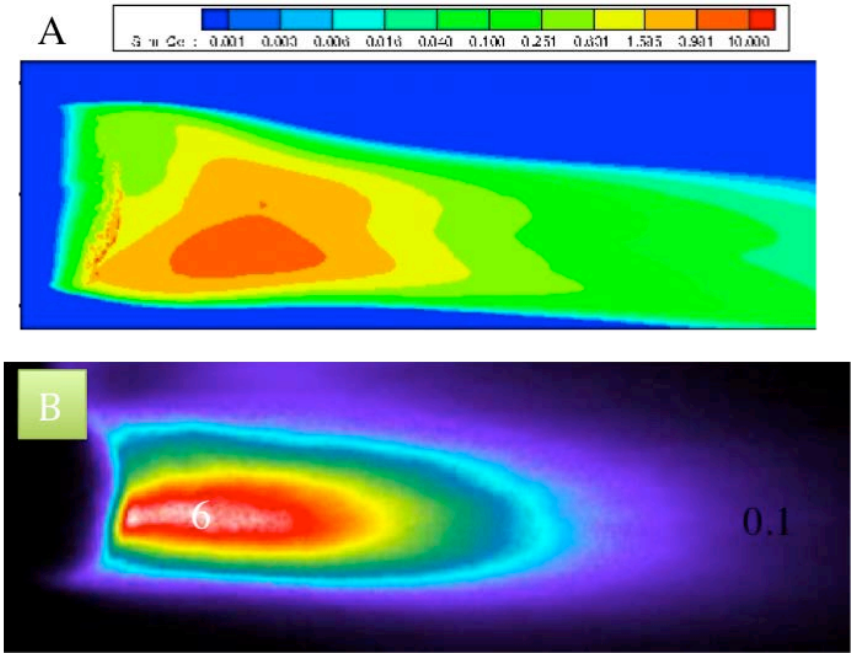

Figure 4. Contour plots for the bioluminescence signature of a 32 mm diameter sphere, moving to the left at a speed of 1 m/s. (A) Predicted integrated biolum-inescence, expressed as cells/ml, for a cell concentration of 32 cells/ml of **Lingulodinium polyedrum**, *based on on the computed probability of stimulation (Fig. 3C). (B) Measured bioluminescence, shown in false color, for a cell concentration of 32 cells/ml of* **L. polyedrum**, *for an average of 5 video frames, each 50 ms in duration. Numbers are in units of cells/ml. Predicted bioluminescence in (A) was within a factor of 2 of measured bioluminescence.*

1.2. High-resolution computations of the flow over a 15 cm long 6:1 ellipsoid were not useful. In an effort to reduce computational cost, symmetry was utilized and that appears to have dampened the formation of turbulent flow. Because of the very high resolution used in this grid, the maximum time-step was 2×10^{-6} seconds (i.e., 2 μs) (for comparison, the sphere grid required a time-step of 5×10^{-5} s (50 μs) and a very long time (calendar and computational) was required to reach a stationary solution and realize the error.

2. Laboratory characterization of two bathyphotometer flow agitators (NOSC and BIOLITE) used by naval oceanographers revealed the presence of a critical volume flow rate, above which bioluminescence potential remained nearly constant and scaled with dinoflagellate cell concentration. The NOSC flow agitator had dominant secondary recirculating flows that were the origin of most of the stimulated bioluminescence. Tests using natural plankton samples indicated that scaling using bioluminescence potential allows predication of flow-stimulated bioluminescence across different flow fields.

3. Pharmacological treatments of the dinoflagellate *Lingulodinium polyedrum* indicated that cellular regulation of bioluminescence involves a stretch-activated component of the signaling pathway that is hypothesized to be a transient receptor potential (TRP) channel. In other organisms TRP channels are known for their role in the sensing of mechanical stimuli. Understanding the components of the dinoflagellate regulatory pathway for bioluminescence is important for future chemical mitigation strategies involving the inhibition of light production from the source organisms.

IMPACT/APPLICATIONS

Project results will enhance DoD capability for predicting levels of bioluminescence associated with surface and underwater vehicles of naval interest. A coupled BIOSTIM-CFD model can be used to predict signatures in applications involving swimmer delivery vehicles and other submersible platforms, as well as torpedoes and other high-speed objects. The breakthrough in providing this capability is the development and application of the BIOSTIM model, developed by PI's Deane and Stokes, that forms a theoretical basis for studying the relationship between flow stimulation and the bioluminescence response. The BIOSTIM model, when coupled to computational hydrodynamics models that provides values of shear stress for a given flow field, allows for predictions bioluminescence intensity for a given level of bioluminescence potential, either measured directly or obtained from the NAVOCEANO METOC database once a transfer function between the flow agitator and flow field is known.

Bioluminescence potential, used to scale model predictions of bioluminescence signatures, is measured by bathyphotometers such as those used by the Naval Oceanographic Office (NAVOCEANO). The usefulness of these measurements has been limited by the difficulty in comparing results from different bathyphotometer systems. Our characterization study of the NOSC and BIOLITE flow agitators provides a context for inter-instrument comparisons that are best done in a controlled laboratory setting under prescribed conditions of known species, cell concentration, and volume flow rate. As each bathyphotometer system is unique in terms of flow pattern, residence time, efficiency of stimulation, measurement geometry, and detector type, a comprehensive evaluation under laboratory conditions will permit the establishment of a suitable transfer function to permit direct comparison of bathyphotometer measurements.

A coupled BIOSTIM-CFD model introduces a new predictive capability for estimated bioluminescence signatures. A validated model can then be verified with full-scale experiments with surface ships and underwater vehicles of naval interest. In situations where field tests are not possible, once a transfer function between the flow agitator and flow field is known, it can be used with the NAVOCEANO METOC database of bioluminescence potential measurements to predict bioluminescence signatures in essentially any oceanic region. The Non-acoustical Optical Vulnerability Assessment Software (NOVAS) being developed by NRL (Matulewski and McBride 2005) has a placeholder in which the coupled BIOSTIM-CFD model can be incorporated into the nighttime visibility assessment component.

This work on predicting bioluminescence signatures is part of a broader program related to bioluminescence that also involves work on mitigation strategies for reducing bioluminescence signatures. One approach is based on chemical inhibition of bioluminescence expression from the source organisms. Our work in understanding the cellular regulation of dinoflagellate bioluminescence is a step in identifying the biochemical and molecular components of the bioluminescence regulatory system in dinoflagellates, the most important source of bioluminescence in littoral and surface waters.

RELATED PROJECTS

The objectives of this project are complimentary and related to the objectives of an NSF funded project to better understand energy dissipation within breaking wave crests using the bioluminescent flash response of dinoflagellates as a flow visualization tool.

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